

WHAT IS CLAIMED IS:

- Donor
Event
Claims
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1. 1. An isolated DNA molecule selected from the group consisting of:
 - 2 A. the DNA sequence of FIGURE 1 (SEQ ID NO:1);
 - 3 B. the DNA sequence of FIGURE 2 (SEQ ID NO:3);
 - 4 C. the DNA sequence of FIGURE 20A (SEQ ID NO:22);
 - 5 D. DNA sequences that hybridize to any of the foregoing DNA sequences under
 - 6 standard hybridization conditions;
 - 7 E. DNA sequences that code on expression for an amino acid sequence encoded
 - 8 by any of the foregoing DNA sequences;
 - 9 F. degenerate variants thereof;
 - 10 G. alleles thereof; and
 - H. hybridizable fragments thereof.
 - 1 2. An isolated nucleic acid molecule, which nucleic acid molecule encodes an
 - 2 ob polypeptide, which polypeptide is characterized by having about 145 to about
 - 3 167 amino acid residues, being expressed predominantly by adipocytes, and being
 - capable of inducing a reduction of body weight in an animal.
 - 1 3. The isolated nucleic acid of Claim 2, wherein the ob polypeptide has an
 - 2 amino acid sequence selected from the group consisting of the sequence depicted in:
 - 3 a) Figure 1 (SEQ ID NO:2),
 - 4 b) Figure 1 from amino acid number 22 to amino acid number 167, Figure 3
 - 5 (SEQ ID NO:4),
 - 6 c) Figure 3 from amino acid number 22 to amino acid number 167,
 - 7 d) Figure 5 (SEQ ID NO:5),
 - 8 e) Figure 5 from amino acid number 22 to amino acid number 166,
 - 9 f) Figure 6 (SEQ ID NO:6), and
 - g) Figure 6 from amino acid number 22 to amino acid number 166.

- 1 4. The nucleic acid molecule of Claim 2 selected from the group consisting of DNA and RNA.
- 1 5. The nucleic acid molecule of Claim 2, which has a sequence as shown in Figure 1 (SEQ ID NO:1) from nucleotide number 46 to nucleotide number 550.
- 1 6. The nucleic acid molecule of Claim 2, which has a sequence as shown in Figure 2 (SEQ ID NO:2) from nucleotide number 46 to nucleotide number 550.
7. The nucleic acid molecule of Claim 1 which is detectably labeled.
8. A vector, which comprises the DNA molecule of Claim 1.
- 1 9. An expression vector, which comprises the nucleic acid molecule of Claim 2, operatively associated with an expression control sequence.
- 1 10. A nucleic acid hybridizable to a non-coding region of an *ob* nucleic acid, which non-coding region is selected from the group consisting of an intron, a 5' non-coding region, and a 3' non-coding region.
- 1 11. A probe capable of screening for a nucleic acid encoding an *ob* polypeptide, which probe is a labeled DNA molecule of Claim 1.
12. A unicellular host transfected with a cloning vector of Claim 8.
13. A unicellular host transfected with an expression vector of Claim 9.
- 1 14. The unicellular host of Claim 13 wherein the unicellular host is selected from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, *Pichia*

3 yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, and BMT10 cells,
plant cells, insect cells, and human cells in tissue culture.

1 15. An oligonucleotide primer for amplifying human genomic DNA encoding an
ob polypeptide.

1 16. The oligonucleotide of Claim 15, which is selected from the group consisting
2 of

3 HOB 1gF 5'-CCCAAGAAGCCCATCCTG-3' (SEQ ID NO:26)

4 HOB 1gR 5'-GACTATCTGGGTCCAGTGCC-3' (SEQ ID NO:27)

5 HOB 2gF 5'-CCACATGCTGAGCACTTGT-3' (SEQ ID NO:28)

HOB 2gR 5'-CTTCAATCCTGGAGATACTGG-3' (SEQ ID NO:29).

1 17. An ob polypeptide, which polypeptide is encoded by the DNA molecule of
Claim 1.

1 18. An ob polypeptide, which polypeptide is characterized by having about 145
2 to about 167 amino acid residues, being expressed predominantly by adipocytes,
and being capable of inducing a reduction of body weight in an animal.

1 19. The ob polypeptide of Claim 18 which has the amino acid sequence shown
in Figure 1 (SEQ ID NO:2) or Figure 5 (SEQ ID NO:5).

1 20. The ob polypeptide of Claim 19 which has the amino acid sequence shown
in Figure 3 (SEQ ID NO:4) or Figure 6 (SEQ ID NO:6).

1 21. An immunogenic fragment of an ob polypeptide, which polypeptide is
2 characterized by having about 160 amino acid residues, being expressed
3 predominantly by adipocytes, and being capable of inducing a reduction of body
weight in an animal.

- 1 22. The immunogenic fragment of an ob polypeptide of Claim 21, which is
- 2 selected from the group consisting of .
- 3 Val-Pro-Ile-Gln-Lys-Val-Gln-Asp-Asp-Thr-Lys-Thr-Leu-Ile-Lys-Thr (SEQ ID
- 4 NO:18);
- 5 Leu-His-Pro-Ile-Leu-Ser-Leu-Ser-Lys-Met-Asp-Gln-Thr-Leu-Ala (SEQ ID
- 6 NO:19);
- 7 Ser-Lys-Ser-Cys-Ser-Leu-Pro-Gln-Thr-Ser-Gly-Leu-Gln-Lys-Pro-Glu-Ser-Leu-
- 8 Asp (SEQ ID NO:20); and
- 9 Ser-Arg-Leu-Gln-Gly-Ser-Leu-Gln-Asp-Ile-Leu-Gln-Gln-Leu-Asp-Val-Ser-Pro-
- Glu-Cys (SEQ ID NO:21).

- 1 23. A derivative of a polypeptide according to claim 17 or 18 having one or
more chemical moieties attached thereto.

- 1 24. The derivative of claim 15 wherein the chemical moiety is a water soluble
polymer.

- 1 25. The derivative of claim 16 wherein the water soluble polymer is
polyethylene glycol.

- 1 26. An analog of an ob polypeptide having the amino acid sequence of human ob
depicted in Figure 4, which analog is selected from the group consisting of:
 - 3 A. serine residue at position 53 substituted with glycine, alanine, valine,
4 cysteine, methionine, or threonine;
 - 5 B. serine residue at position 98 substituted with glycine, alanine, valine,
6 cysteine, methionine, or threonine;
 - 7 C. arginine residue at position number 92 substituted with asparagine, lysine,
8 histidine, glutamine, glutamic acid, aspartic acid, serine, threonine, methionine, or
9 cysteine;
 - 10 D. one or more of residues 121 to 128 substituted with glycines or alanines;

- 11 E. deletion of one or more amino acid residues at positions 121-128;
 - 12 F. a loop structure formed by the disulfide bond that forms between cysteine
 - 13 residues 117 and 167;
 - 14 G. amino acids from residue 22 to 53;
 - 15 H. amino acids from residue 61 to amino acid residue 116;
 - 16 I. amino acids from residue 61 to amino acid residue 167;
 - 17 J. aspartic acid at one or more of residues 29, 30, 44, 61, 76, 100, and 106
 - 18 substituted with glutamic acid; and
- K. one or more isoleucine residues substituted with leucine.

1 27. A method for preparing an ob polypeptide comprising:
2 A. culturing a unicellular host of Claim 12 or 13 under conditions that provide
3 for expression of the ob polypeptide; and
4 B. recovering the expressed ob polypeptide.

28. The method according to Claim 27 wherein the host cell is a bacterium.

29. The method according to Claim 27, wherein the host cell is a yeast.

1 30. The method according to Claim 27, further comprising:
2 C. chromatographing the polypeptide on a Ni-chelation column; and
3 D. purifying the polypeptide by gel filtration.

1 31. The method according to Claim 30, further comprising after step C and
2 before step D chromatographing the ob polypeptide on a strong cation exchanger
3 column.

32. An antibody to the ob polypeptide of Claim 17.

33. An antibody to the ob polypeptide of Claim 18.

- 1 34. A method for preparing an antibody to an ob polypeptide, comprising:
 - 2 A. conjugating the immunogenic fragment of an ob polypeptide of Claim 19 to
 - 3 a carrier protein;
 - 4 B. immunizing a host animal with the ob polypeptide fragment-carrier protein
 - 5 conjugate of step A admixed with an adjuvant; and
 - 6 C. obtaining antibody from the immunized host animal.
- 7
8 35. The antibody of Claim 32, 33, or 34 which is a polyclonal antibody.
- 9
10 36. The antibody of Claim 32, 33, or 34 which is a monoclonal antibody.
- 11
12 37. An immortal cell line that produces a monoclonal antibody according to
- 13 Claim 36.
- 14
15 38. The antibody of Claim 32, 33, or 34 labeled with a detectable label.
- 16
17 39. A method for measuring the presence of an ob polypeptide in a sample,
18 comprising:
 - 19 A. contacting a sample suspected of containing an ob polypeptide with an
 - 20 antibody that binds to the ob polypeptide under conditions which allow for the
 - 21 formation of reaction complexes comprising the antibody and the ob polypeptide,
 - 22 B. detecting the formation of reaction complexes comprising the antibody and
 - 23 ob polypeptide in the sample;
 - 24 in which detection of the formation of reaction complexes indicates the presence of
 - 25 ob polypeptide in the sample.
- 26
27 40. The method of Claim 39 in which the antibody is bound to a solid phase
- 28 support.

1 41. A method for evaluating the level of ob polypeptide in a biological sample
2 comprising

3 A. detecting the formation of reaction complexes in a biological sample
4 according to the method of Claim 30; and

5 B. evaluating the amount of reaction complexes formed, which amount of
6 reaction complexes corresponds to the level of ob polypeptide in the biological
sample.

1 42. A method for detecting or diagnosing the presence of a disease associated
2 with elevated or decreased levels of ob polypeptide in a mammalian subject
3 comprising:

4 A. evaluating the level of ob polypeptide in a biological sample from a
5 mammalian subject according to Claim 41; and

6 B. comparing the level detected in step (A) to a level of ob polypeptide present
7 in normals or in the subject at an earlier time;
8 in which an increase in the level of ob polypeptide as compared to normal levels
9 indicates a disease associated with elevated levels of ob polypeptide, and decreased
10 level of ob polypeptide as compared to normal levels indicates a disease associated
with decreased levels of ob polypeptide.

1 43. A method for monitoring a therapeutic treatment of a disease associated with
2 elevated or decreased levels of ob polypeptide in a mammalian subject comprising
3 evaluating the levels of ob polypeptide in a series of biological samples obtained at
4 different time points from a mammalian subject undergoing a therapeutic treatment
5 for a disease associated with elevated or decreased levels of ob polypeptide
according to the method of Claim 41.

1 44. A method for changing the body weight of a mammal comprising inhibiting
the expression of an ob polypeptide encoded by a nucleic acid of Claim 2.

- 1 45. The method according to Claim 44 comprising expressing an antisense
2 nucleic acid molecule hybridizable to a nucleic acid that expresses the ob
3 polypeptide, expressing a ribozyme that cleaves a nucleic acid that expresses the ob
4 polypeptide, administering an antisense nucleic acid molecule hybridizable to a
5 nucleic acid that expresses the ob polypeptide, and administering a ribozyme that
 cleaves a nucleic acid that expresses the ob polypeptide.

- 1 46. A pharmaceutical composition for reducing body weight of an animal
2 comprising the ob polypeptide of Claim 17 and a pharmaceutically acceptable
 carrier.

- 1 47. A pharmaceutical composition for reducing body weight of an animal
2 comprising the ob polypeptide of Claim 18 and a pharmaceutically acceptable
 carrier.

- 1 48. A method for reducing the body weight of an animal comprising
2 administering an amount of a pharmaceutical composition of Claim 47 effective to
3 reduce the body weight of an animal to an animal believed to be in need of
 decreased body weight.

- 1 49. The method according to Claim 48 wherein the animal is a human, and the
 ob polypeptide is human ob polypeptide.

- 1 50. A method for reducing the body weight of a mammal comprising increasing
 the expression of a protein encoded by the nucleic acid of Claim 2.

- 1 51. A pharmaceutical composition for increasing the body weight of an animal
 comprising an antagonist of an ob polypeptide.

- 1 52. The pharmaceutical composition of Claim 51, wherein the antagonist is
 - 2 selected from the group consisting of an antibody that binds to and neutralizes the
 - 3 activity of ob polypeptide, a fragment of the ob polypeptide that binds to but does
 not activate the ob receptor, and a small molecule antagonist of the ob polypeptide.
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- 1 53. A method for increasing the body weight of an animal comprising
 - 2 administering an amount of the pharmaceutical composition of Claim 51 effective to
 - 3 cause an increase in body weight to an animal believed to be in need of increased
 body weight.